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SHORT NOTE

Internal Armature of the Hindgut of *Pericapritermes nitobei* (Shiraki)

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Abstract

The internal armature of the hindgut of *Pericapritermes nitobei* was examined under a light microscope. Termites were obtained from Iriomote Island, Okinawa Prefecture, Japan, identified based on the typological characters of soldiers and 12S (mitochondrial) ribosomal RNA sequencing, and dissected to examine hindgut morphology. The hindgut of *P. nitobei* could be separated into five parts, P1-P5, and P1-P3 had cuticular spines on the inner surface. P1 bore small triangular spines. P2 formed an enteric valve, composed of six finger-shaped enteric pads with triangular spines, and six marginal regions with small dot-like spines. P3 bore three different types of spines: relatively large star-shaped spines; thorn-like small spines, and long curved spines with brush-like tips.

Many subterranean termite species have a cuticular armature on the internal surface of the digestive tract; this is assumed to serve as a valvular apparatus, which is generally termed an “enteric valve” (Noirot & Noirot-Timothee, 1969). Valve morphology differs among species, and is sometimes used as an identification and/or taxonomic character (e.g., Grassé & Noirot, 1954). Notably, the structure is extensively divergent in soil-feeding species, and is an important taxonomic character for soldierless termites (e.g., Sands, 1972; Fontes, 1992; Sands, 1998; Noirot, 2001; Donovan, 2002).

Two species of soil-feeding termites, *Pericapritermes nitobei* (Shiraki) and *Sinocapritermes mushae* (Oshima and Maki), have been reported from Japan (Ikehara, 1957; Takematsu, 1994). However, the detailed characteristics of their enteric valves have not yet been examined. In the present study, we examined the enteric valve and the other hindgut armature of *P. nitobei*.

Two colonies of *P. nitobei* were obtained from soil of Iriomote Island, Okinawa Prefecture, Japan (location: 24°36'33" N, 123.79°61" E, 27.3 m a.s.l.) on June 5 2013. The

colonies were fixed on-site in absolute ethanol and brought back to the laboratory. The species was identified based upon the external morphology of soldiers and the sequence of the mitochondrial 12S ribosomal RNA of workers. Soldiers were transferred to 70% ethanol, and observed under a dissection microscope (Leica S8 Apo: Leica Japan, Tokyo, Japan). We initially focused on head and mandible morphology, and compared our data to those of a previous study (Yasuda et al., 2000). Several workers were next dissected to observe the hindgut structure. The workers were transferred to 70% ethanol, and entire digestive system was extracted with tweezers and insect pins, and the hindgut elements were dissected separately using sharpened insect pins. The dissected elements were mounted using 70% ethanol, and photomicrographs were taken using a digital camera (DXM1200: Nikon, Tokyo, Japan) fitted to a light microscope facilitated with a DIC optics (Nikon Eclipse 80i: Nikon, Tokyo, Japan). The head capsule was transferred to PrepMan™ Ultra DNA sample preparation reagent (Applied Biosystems, Foster City, CA), digested following the manufacturer's instructions, and used



as a PCR template. PCR amplification and sequencing were performed as described previously (Inward et al., 2007).

The termites were identified as *P. nitobei*, the only *Pericapritermes* species reported from Japan, because the soldiers had a strongly curved left mandible and a long and round-to-square head. The sequence of the 12S mitochondrial RNA of dissected workers was identical to the *P. nitobei* sequence stored in GenBank (accession numbers: AB006584 and DQ441790).

The hindgut of *P. nitobei* was clearly separable into five parts, P1 (= ileum), P2 (= enteric valve), P3 (paunch), P4 (= colon) and P5 (= rectum). P1 was relatively tube-like, followed by a narrow constriction (P2). P2 formed an enteric valve, and the hind gut of ethanol-fixed material was often broken at the posterior end of P2 (= the P2/P3 junction). P3 was expanded to form a large sac, narrowing at the posterior end, and smoothly connected to a tube-like colon (= P4) which is smoothly connected to a little expanded short rectum (= P5). Of these five sections, P4 and P5 had no obvious internal armature, but the other three parts bore various forms of cuticular structures. The locations of the various types of hindgut spines in P1-P3 are shown schematically in Figure 1.

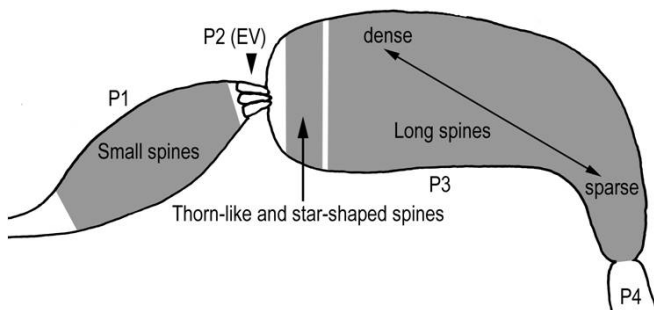


Fig 1. Schematic drawing of the hindgut of *Pericapritermes nitobei*. The sections are indicated as P1, P2 (EV), P3, and P4. The locations and densities of spines are coded using a grayscale and are indicated by arrows. P4 and P5 are not drawn because these parts do not have cuticular spines.

The internal surface of P1 bore amber-colored triangular spines, which were relatively simple and small (Fig 2). The spinal arrangement seemed to be irregular, and the spines occupied almost all of P1. P2 formed a moderately developed enteric valve, separable into six enteric pads and six marginal regions. The enteric pads were finger-shaped (i.e., with rounded posterior ends) and bore light amber-colored triangular spines (Fig 3). The tips of the spines were relatively blunt, forming somewhat flattened triangles (Fig 3). The marginal parts were membrane-like, with small dot-like cuticular spines (Fig 3). Compared to some other *Pericapritermes* species, the enteric valve of *P. nitobei* was somewhat similar to that of *P. papuanus* Bourguignon, Leponce & Roisin 2008. Thus, *P. papuanus* bears triangular spines on enteric pads, and dot-like cuticles on the marginal regions, although the shape of the triangular spines and the density of dots differ from those of the species under discussion in the present report (Bourguignon et al., 2008).

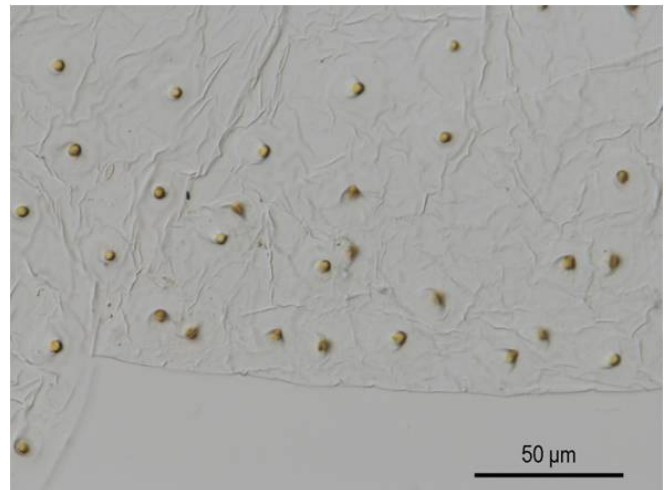


Fig 2. Internal armature of the P1 section of *Pericapritermes nitobei*.

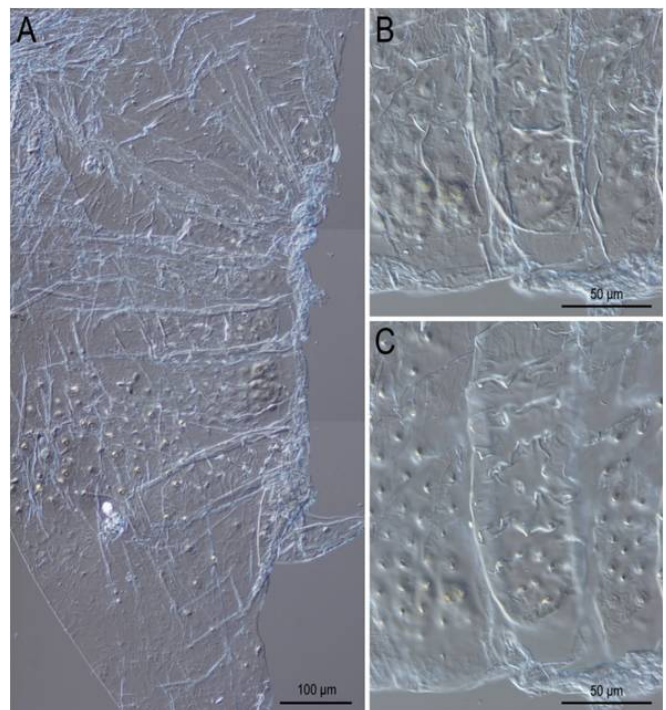


Fig 3. The enteric valve (P2 section) of *Pericapritermes nitobei*. A: An entire enteric valve was expanded and mounted on a slide; B, C: An enteric pad (center) and marginal regions (right and left sides) in different focal planes.

The internal armature of P3 was of interest. Three different types of spine were evident. These were large, dark, amber-colored star-shaped spines formed by two palm-like components adherent at their bases (Fig. 4A, B); small, light amber-colored thorn- or arrowhead-like spines (Fig. 4C); and long, dark, amber-colored curved spines with brush-like split tips (Fig. 4D). The positions of these spines within P3 were distinct. The thorn-like and star-shaped spines were located ~20% of the total P3 length from the anterior end. Although the detailed arrangements could not be clearly observed, these two types of spine occurred together and formed a narrow spiny band. The long spines covered the remainder of P3, but were most dense in the anterior and middle regions, becoming

gradually sparser posteriorly. Brown glue-like materials, assumed to be organic in nature, were adherent to the bases of the long spines and were evident in all P3 regions bearing such spines. The organic material could be removed by sonication, but not by tweezers or insect pins. Thus, the spines, especially the long spines, may trap organic nutrient materials.

The structures and arrangement of spines were consistent among all individual workers examined. We did not examine soldier structure because only one soldier was obtained from each colony. More soldier materials are required for detailed observation.

The enteric valve structure of higher termites has become an important taxonomic character (Donovan, 2002; Bourguignon et al., 2008; Scheffrahn, 2013). Scheffrahn (2013) reported small spiny flap-like cuticular structures on the posterior end of the enteric valve seat of *Compositermes vindai*.

Also, Bignell et al. (1980, 1983) described long spines in the P3 regions of *Procubitermes aburiensis* and *Cubitermes severus*. Further, Noirot (2001) photographed a row of saw blade-like spines bearing of P3 of *P. magnificus* which is clearly different from any of P3 spines of *P. nitbei*. Homology among those cuticular structures and the structures found in the present study are necessary to be examined in the future with developmental and/or molecular works, e.g., analysis of tissue-specific gene expression pattern. However, in most instances, the P1 and P3 sections of termite guts have been inadequately studied. Nevertheless, such characters may be useful in terms of functional morphology and termite taxonomy, and warrant close examination.

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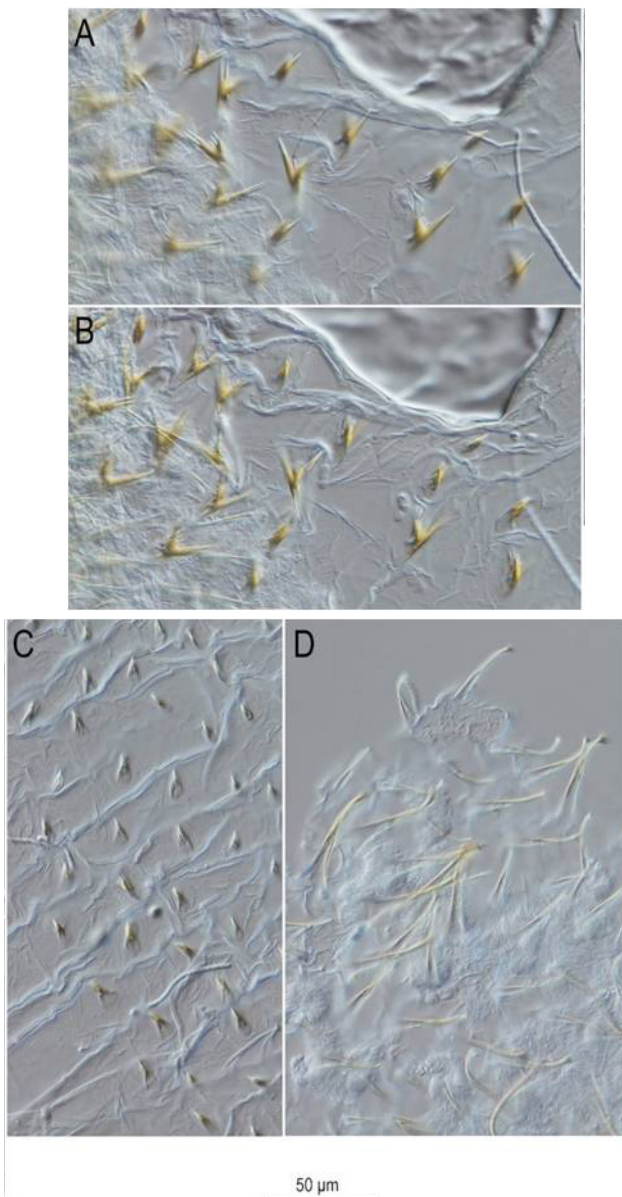


Fig 4. Internal armature of the P3 section of *Pericapritermes nitobei*. A, B: Star-shaped spines in different focal planes; C: Thorn-like spines; D: Long curved spines.

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